AD_____

Award Number: W81XWH-12-1-0072

TITLE: Molecular Heterogeneity in Primary and Metastatic Prostate Tumor Tissue

PRINCIPAL INVESTIGATOR: Dr. Julie Batista

CONTRACTING ORGANIZATION: Harvard College
BOSTON MA 02115-6028

REPORT DATE: October 2014

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
October 2014	Annual Summary	30 Sep 2013 – 29 Sep 2014

4. TITLE AND SUBTITLE
Molecular Heterogeneity in Primary Metastatic Prostate Tumor Tissue

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0072

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Julie Batista

E-mail: jkasperz@hsph.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Harvard College

Boston, MA 02115-6028

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: The overarching goal of the grant is to characterize molecular heterogeneity in multi-focal and metastatic prostate cancer. Aim 1 focuses on a 4-gene signature of prostate cancer prognosis, and whether the signature differs across within-patient tumor nodules. Aim 2 compares gene expression profiles between primary and lymph node metastases in order to identify genes involved in metastatic progression of prostate cancer. Scope: In year 1, Dr. Batista has received IRB approval, completed a series of courses to augment her expertise in prostate cancer epidemiology, has coordinated meetings to discuss the study progress with collaborators, and has begun specimen and data collection for the proposed work. In the upcoming year 2, Dr. Batista will complete data collection, lead the statistical analyses, and publish the findings in peer-reviewed journals. Major Findings: To date, the proposed biomarkers are in the process of being measured. In a related analysis of tumor expression of prostate-specific membrane antigen (PSMA) and prostate cancer-specific mortality, Batista et al. found that PSMA was positively correlated with Gleason score and tumor angiogenesis, but was not an independent predictor of prostate cancer survival (*Cancer Epidemiol Biomarkers Prev*, in press). Significance: The clinical significance of the project is to better characterize putative prognostic markers for prostate cancer, as well as identify potential therapeutic targets for secondary prevention.

15. SUBJECT TERMS
Prostate cancer, metastases, prognosis, heterogeneity, gene expression

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	12	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Page

INTRODUCTION

Since approximately 1 in 8 men with prostate cancer in the US will die of their disease, it is critical to identify early in the disease course those men who are likely to progress in order to administer appropriate therapies. Several tumor-derived RNA expression signatures have been developed to improve upon the prognostic value of known clinical parameters (e.g. Gleason score, tumor stage, PSA levels) to predict prostate cancer recurrence or death. However, hundreds of genes have been identified in the current signatures, and it is unclear which ones are biologically relevant for metastatic spread due, in part, to the difficulty in obtaining metastatic specimens and inherent tumor heterogeneity. The focus of the original proposal was to assess tumor heterogeneity and prognostic value of a 4-gene signature of prostate cancer prognosis,[1] and to identify genes involved in metastatic progression to the lymph nodes.

The current progress report focuses on tumor heterogeneity of one of the four genes: Phosphatase and tensin homolog (PTEN). PTEN is a well-known tumor suppressor gene that acts as a negative regulator of the PI3K/AKT pathway. Loss of PTEN expression has been associated with aggressive prostate cancer and adverse outcomes in several studies.[2,3] Since PTEN expression appears to have prognostic utility and may guide treatment decisions, it is important to characterize heterogeneity as prostate biopsies sample only a portion of the existing tumor. However, detailed characterization of PTEN heterogeneity within and between tumor foci in prostate cancer patients is limited. To our knowledge, only one prior study has assessed the distribution of PTEN loss in multifocal prostate cancer: among 142 patients that underwent radical prostatectomy, PTEN deletion was observed in 42% of patients and was significantly correlated with higher tumor Gleason grade. [4] Our study is slightly larger and assesses PTEN protein loss using an alternative, valid method of immunohistochemistry.[5,6] Furthermore, we are able to link PTEN loss with long-term clinical outcomes.

BODY

Task 1. Aim 1: Characterize heterogeneity of a 4-gene signature across prostate tumor nodules and validate its prognostic potential

IRB approval was obtained for this project at Harvard School of Public Health in October 2012. The tissue microarray of approximately 200 prostate cancer patients that underwent radical prostatectomy, including approximately one third with multi-focal disease, was constructed in 2013. Since the original grant proposal, the funding source to measure the 4-gene signature in these tumor specimens became unavailable to perform the assay.[1] In year 2, Dr. Batista secured an alternative source to measure one of the genes (PTEN) in the laboratory of Dr. Tamara Lotan at Johns Hopkins School of Medicine. Dr. Lotan is an expert pathologist and has developed an immunohistochemical method for measuring PTEN expression that is valid and methodologically easier than the alternative FISH assay.[5,6] The following are our current findings on intratumoral heterogeneity of PTEN staining in a Swedish cohort of prostate cancer patients.

PTEN was evaluated in archival tumor tissue from 197 prostate cancer patients diagnosed from 1989-2005 (**Table 1** in Supporting Data). A single tumor focus was evaluated for

PTEN protein expression in 132 (67%) of patients, while 2-4 tumor foci were evaluated in 65 (33%) of patients. PTEN loss was assigned if the patient had any areas of the tumor showing markedly decreased or completely negative immunohistochemical staining (at least 5% of cells), as compared with benign epithelium and stromal cells within the tumor. A patient was scored as PTEN heterogeneous if a portion of the tumor had PTEN loss and another portion did not. We found that PTEN loss in ≥1 tumor core was present in 74 (38%) of patients, and that consistent PTEN loss across all cores evaluated was present in 23 (12%) of patients (**Table 2** in Supporting Data). This is in agreement with Yoshimoto et al. who found that PTEN deletion was present in 42% of prostate cancer patients,[4] whereas Gumuskaya et al. noted PTEN loss in 53% of patients.[6] In our study, heterogeneity in PTEN expression within a tumor focus was noted in 44 (22%) of patients, and heterogeneity between tumor foci was noted in 26 of the 65 patients (40%) with >1 tumor focus evaluated.

Among the 197 patients, 250 individual tumor cores were individually evaluated for PTEN expression and Gleason score. PTEN loss was significantly correlated with higher Gleason score, with 57% of Gleason score ≥8 cores showing PTEN loss compared to 11% of Gleason score ≤6 cores (**Figure 1** in Supporting Data). Within-core heterogeneity was also positively correlated with Gleason score, but to a lesser degree. PTEN loss in all tumor cores was associated with a non-significant increase in prostate cancer-specific (hazard ratio (HR)=2.05; 95% confidence interval (CI): 0.58,7.24) and overall (HR=1.56; 95% CI: 0.72,3.36) mortality (**Table 3** in Supporting Data). After adjusting for Gleason score, the associations were attenuated. Heterogeneous loss of PTEN was not associated with mortality. However, with only 14 prostate cancer-specific deaths, we do not have adequate statistical power to assess survival. Thus, we are currently collecting information on biochemical recurrence and progression to castrate resistant disease from medical records; this additional information will be available in November 2014.

Task 2. Aim 2: Identify genes critical for metastatic progression to lymph nodes in prostate cancer

IRB approval was obtained at Harvard School of Public Health in October 2012. Dr. Batista has performed a literature review detailing current studies that have compared molecular differences in metastatic versus primary prostate cancer. She has also taken several courses to enrich her knowledge of pathology, molecular epidemiology, and biostatistics (see Task 4 below). These courses have aided Dr. Batista in the study design of the project, and will also be key to the statistical analysis.

Our collaborator, Dr. Ove Andren, has recently finished the tissue collection of within-person primary and lymph node-positive archival tumor specimens in Sweden. Of the hundreds of records reviewed, 5 patient-matched radical prostatectomy and positive lymph node samples were identified. This is less than the expected number of 10-15 matched pairs. However, Dr. Andren was able to identify an addition 50 patients with positive lymph nodes for which the diagnostic biopsy specimen is available for analysis. Since biopsy specimens have very small amounts of tumor tissue, and thus low yields of mRNA, we are actively devising a feasible plan to best address these methodological challenges for mRNA expression profiling.

Task 3. Mentored training with Dr. Mucci

Drs. Mucci and Batista have completed this task by meeting regularly to discuss progress on the specific aims of the project, as well as evaluating short- and long-term goals.

Task 4. Coursework

In year 1, Dr. Batista took several courses in pathology, molecular epidemiology, and biostatistics. In September 2012, Dr. Batista attended a 2-hr course on "Introduction to Microarrays and Affymetrix Data analysis using R/Bioconductor" at Harvard Medical School where she familiarized herself with the R programming language. In October 2013, Dr. Batista attended a 2-hr course on "Whole Transcript Expression analysis using Gene and Exon 1.0 ST arrays" at Harvard Medical School. The course further developed her knowledge of the R programming language and techniques for analyzing expression array data. In January 2013, Dr. Batista completed EPI508 (Pathology for Epidemiologists; 1-week course) with a grade of 'Pass' at Harvard School of Public Health. The objective of the course was to provide an overview of tumor classification systems, histology, immunohistochemistry, and other molecular techniques used in epidemiologic research involving tumor specimens. From January-May 2013, Dr. Batista completed BIO508 (Genomic Data Manipulation; semester-long course) at Harvard School of Public Health with a grade of "A." The course taught computational methods for genomic data analysis using the Python programming language and online, publically available research tools. All formal coursework was completed in year 1.

In year 2, Dr. Batista continued her training by attending an "Introduction to Network Medicine" course (October 2013) hosted by the Harvard Catalyst. The 3-day course provided an introduction to the identification and investigation of molecular networks that underlie disease etiology and treatment.

Task 5. Meetings and seminars

Dr. Batista has attended numerous meetings and seminars as planned in years 1 and 2. She has attended two bi-weekly meetings, including a prostate cancer epidemiology meeting and pathology-epidemiology working group. Monthly meetings that Dr. Batista attends include meetings for the Prostate Cancer SPORE at Dana-Farber/Harvard Cancer Center and for prostate cancer journal club at Harvard School of Public Health. Dr. Batista also took part in a special week-long workshop entitled "Integrative Molecular Epidemiology Workshop" in July 2013 in Boston, MA, sponsored by the American Association of Cancer Research. This workshop addressed the challenges faced when integrating high-dimensional data from multiple sources in order to inform disease etiology and outcomes. In March 2013 and March 2014, Dr. Batista presented an abstract at the Multi-Institutional Prostate Cancer Program Retreat in Ft. Lauderdale, Florida. She also presented a poster at the American Institute for Cancer Research annual conference in November 2013 in Washington, DC.

KEY RESEARCH ACCOMPLISHMENTS

- Publication of four co-authored manuscripts in peer-reviewed journals [7-10]
- Literature review of current studies comparing molecular differences in metastatic versus primary prostate cancer
- Completion of a tissue microarray with prostate tumor specimens representing patients with multi-focal disease
- Completion of initial statistical analysis and manuscript preparation for Aim 1
- Development of a prostate tumor tissue resource that utilizes patient-matched primary and lymph node-positive prostate cancer specimens

REPORTABLE OUTCOMES

- Dr. Batista was promoted to Instructor in the Department of Medicine at Harvard Medical School/Brigham and Women's Hospital (July 2013)
- Publication of four manuscripts in peer-reviewed journals from 2013-2014 [7-10]
- Became co-investigator on funded R01 project (PI: Massimo Loda, Dana-Farber Cancer Institute) entitled "Molecular link between metabolic syndrome and prostate cancer."
- Became co-investigator on funded Dana-Farber Cancer Institute, A. David Mazzone Disparity Research Award (PI: Mark Preston, Brigham and Women's Hospital) entitled "Do baseline prostate specific antigen (PSA) levels predict advanced prostate cancer in African American men?"
- Applied for Prevent Cancer Foundation award (PI: Julie Batista, Brigham and Women's Hospital) in August 2014 entitled "Dairy intake in adolescence/adulthood and advanced prostate cancer risk"; awaiting grant review.
- Presentation of abstracts at the Multi-Institutional Prostate Cancer Program Retreat in Ft. Lauderdale, Florida (March 2013 and March 2014) and the American Institute for Cancer Research annual conference in Washington, DC (November 2013).
- Completion of a tissue resource by colleague (Dr. Ove Andren) that utilizes tissue microarray technology to catalog >200 prostate cancer patients with single and multi-focal prostate tumor specimens. These tissue microarrays were used for the analyses in Aim 1, and are available as a resource for any of our collaborators who wish to study protein expression and histological differences across tumor foci in this patient population. (2013-2014)
- Development of a tissue resource that combines within-person primary and lymph node-positive prostate cancer specimens. This resource is coordinated in Sweden by Dr. Ove Andren and the archival tumor specimens are readily available for research purposes (Aim 2).

CONCLUSION

Dr. Batista has made significant progress on the current Career Development Award through coursework, organizational meetings, and completing many research-related tasks. Regarding career accomplishments, Dr. Batista was promoted to Instructor in the Department of Medicine at Harvard Medical School/ Brigham and Women's Hospital in July 2013. Dr. Batista has worked to overcome the challenge of finding an alternative means of performing the assays for Aim 1, and has made great progress to develop the tissue resource for Aim 2. She also published a paper on a putative prognostic marker for prostate cancer in year 1, and has presented her findings at several scientific meetings/conferences. Dr. Batista continues to make significant progress on Aims 1 & 2, and will conclude by submitting her findings to peer-reviewed journals. The current findings on PTEN loss in multifocal prostate cancer, detailed in the Body section of the progress report, highlight that heterogeneous PTEN expression is a common event within and across tumor foci. This information is clinically relevant when evaluating PTEN as a potential prognostic marker in prostate cancer patients. In summary, this project is making important contributions to the understanding and characterization of molecular heterogeneity in prostate cancer.

REFERENCES

1. Ding Z, Wu CJ, Chu GC, Xiao Y, Ho D, Zhang J, Perry SR, Labrot ES, Wu X, Lis R, Hoshida Y, Hiller D, Hu B, Jiang S, Zheng H, Stegh AH, Scott KL, Signoretti S, Bardeesy N, Want YA, Hill DE, Golub TR, Stampfer MJ, Wong WH, Loda M, Chin L, DePinho RA. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. *Nature* 2011;470:269-273.
2. Cuzick J, Yang ZH, Fisher G,Tikishvili E, Stone S, Lanchbury JS, Camacho N, Merson S, Brewer D, Cooper CS, Clark J, Berney DM, Moller H, Scardino P, Sangale Z. Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer. *Br J Cancer* 2013;108:2582-2589.
3. Van der Kwast TH. Prognostic prostate tissue biomarkers of potential clinical use. *Virchows Arch* 2014;464:293-300.
4. Yoshimoto M, Ding K, Sweet JM, Ludkovski O, Trottier G, Song KS, Joshua AM, Fleshner NE, Squire JA, Evans AJ. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. *Mod Pathol* 2013;26:435-447.
5. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, Hicks JL, Park BH, Humphreys E, Partin AW, Han M, Netto GJ, Isaacs WB, DeMarzo AM. PTEN protein loss by immunostaining: Analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res* 2011;17:6563-6573.
6. Gumuskaya B, Gurel B, Fedor H, Tan HL, Weier CA, Hicks JL, Haffner MC, Lotan TL, DeMarzo AM. Assessing the order of critical alterations in prostate cancer development and progression by IHC: further evidence that PTEN loss occurs subsequent to *ERG* gene fusion. *Prostate Cancer Prostatic Dis* 2013;16:209-215.
7. **Kasperzyk JL**, Finn SP, Flavin R, Fiorentino M, Lis R, Hendrickson WK, Clinton SK, Sesso HD, Giovannucci EL, Stampfer MJ, Loda M, Mucci LA. Prostate-specific membrane antigen protein expression in tumor tissue and risk of lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2013;22:2354-63.
8. Russnes KM, Wilson KM, Epstein MM, **Kasperzyk JL**, Stampfer MJ, Kenfield SA, Smeland S, Blomhoff R, Giovannucci EL, Willett WC, Mucci LA. Total antioxidant intake in relation to prostate cancer incidence in the Health Professionals Follow-up Study. *Int J Cancer* 2014;134:1156-65.
9. Preston MA, Riis AH, Ehrenstein V, Breau RH, **Batista JL**, Olumi AF, Mucci LA, Adami HO, Sorensen HT. Metformin use and prostate cancer risk. *European Urology* 2014 Advance online publication. doi: 10.1016/j.eururo.2014.04.027
10. Sigurdardottir LG, Markt SC, Rider JR, Haneuse S, Fall K, Schernhammer ES, Tamimi RM, Flynn-Evans E, **Batista JL**, Launer L, Harris T, Aspelund T, Stampfer MJ, Gudnason V, Czeisler CA, Lockley SW, Valdimarsdottir UA, Mucci LA. Urinary melatonin levels, sleep disruption, and risk of prostate cancer in elderly men. *European Urology* 2014 Advance online publication. doi: 10.1016/j.eururo.2014.07.008

Note. Dr. Batista has published under the last names Kasperzyk and Batista.

SUPPORTING DATA

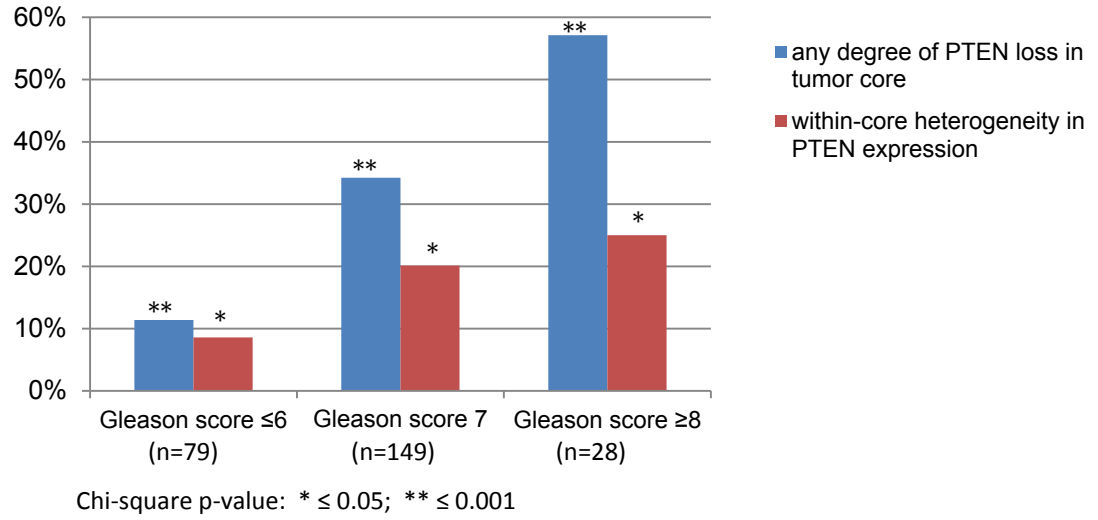
Table 1: Characteristics of 197 prostate cancer patients on tissue microarrays for multi-focal prostate cancer after radical prostatectomy

Mean age at diagnosis, years	62.9
Range of diagnosis years	1989-2005
Mean follow-up time from diagnosis to death or end of follow-up, years	10.7
Number of tumor foci evaluated per patient, %	
1	67%
2	25%
3	7%
4	1%
Clinical T stage, %	
T1a-T1c	55%
T2	37%
T3	4%
Gleason score of primary focus, %	
6	24%
7	64%
8	9%
9	3%
Vital status, N	
Prostate cancer-specific death	14
Death from other cause	29

Table 2: Distribution of PTEN expression in prostate tumor tissue from 197 patients

	N (%)
PTEN consistently expressed in all tumor cores	123 (62%)
PTEN loss in ≥1 tumor core	74 (38%)
PTEN loss in all tumor cores	23 (12%)
Within-focus heterogeneity in PTEN expression (n=197)	44 (22%)
Between-foci heterogeneity in PTEN expression (n=65 multifocal patients)	26 (40%)

Figure 1: Correlation of prostate tumor PTEN loss with Gleason score in individual tumor cores



Chi-square p-value: * ≤ 0.05; ** ≤ 0.001

Table 3: Association of PTEN loss in prostate tumor tissue with overall and disease-specific survival

	PTEN consistently expressed in all tumor cores	Heterogeneous PTEN loss within or across tumor foci	PTEN loss in all tumor cores
Prostate cancer-specific mortality			
N deaths	7	3	4
Age-adjusted HR (95% CI)	1.00 (referent)	0.70 (0.18,2.75)	2.05 (0.58,7.24)
Age- and Gleason score- adjusted HR (95% CI)	1.00 (referent)	0.36 (0.08,1.56)	1.28 (0.35,4.68)
All-cause mortality			
N deaths	22	11	10
Age-adjusted HR (95% CI)	1.00 (referent)	0.84 (0.40,1.77)	1.56 (0.72,3.36)
Age- and Gleason score- adjusted HR (95% CI)	1.00 (referent)	0.71 (0.33,1.51)	1.22 (0.56,2.69)

Abbreviations. Hazard ratio, HR; confidence interval, CI